Olfactory Stimulation of Africanized Honey Bee (Hymenoptera: Apidae) Attacks by Insect Repellents

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ABSTRACT Three common insect repellents (N,N-diethyl-m-toluamide [DEET], Pyranha, and Repel X) were tested to determine whether they affected Africanized honey bee attack behavior. Eight Africanized honey bee (Apis mellifera L.) colonies were exposed in an alternating series to the test repellents or blank controls delivered in a stream of air directed toward the colony entrances. The response generated by the repellents and the controls was measured as the number of attacking honey bees recorded with an electronic temper tester. Neither a citronella-based repellent (Pyranha) nor DEET had any effect on colony behavior; however, Repel X consistently caused a greater attack response after exposure.

KEY WORDS Africanized honey bees, *Apis mellifera*, insect repellents, N,N-diethyl-*m*-toluamide, citronella, attacks

AFRICANIZED HONEY BEES (Apis mellifera L. near scutellata), often colloquially referred to as "killer bees," are best known for their highly defensive behavior, which may culminate in a massive stinging attack (Rodriguez-Lainz et al. 1999, Johnston and Schmidt 2001). In Arizona, three humans and numerous animals including dogs, horses, and at least one cat have been killed by attacks (Johnston and Schmidt 2001). Attacks are provoked by various stimuli including human or animal breath, motion, dark coloration, warmth, large size, and proximity to the colony (reviewed in Schmidt 1998); however, the greatest stimuli for attack appear to be chemical in nature, especially the odors of humans and other animals and their breath (Free 1961, Maschwitz 1964, Schmidt and Boyer Hassem 1996).

Common folklore holds that some animals are more attractive and, therefore, more frequently attacked by bees than others. Horses and dogs are considered to be most attractive animals, a belief at least partially supported by attack statistics (Sugden et al. 1994, Johnston and Schmidt 2001). However, attack figures alone could be misleading: horses and dogs might be attacked more frequently than other animals, such as cows, cats, sheep, or pigs, because of behaviors or

Materials and Methods

Eight feral Africanized honey bee colonies in artificial nest cavities (Schmidt et al. 1989) located in a remote apiary were used for experimentation during July and August 1999. Three insect repellants were tested to determine their ability to stimulate or suppress attack: DEET, the main active ingredient of most insect repellents sold for human use; Pyranha (ChemI-Matic, Houston, TX), a common horse rub that has the smell of citronella and contains pyrethrins, piperonyl butoxide and butoxypolypropylene glycol as active ingredients; and Repel X (Farnam Companies, Phoenix, AZ), a horse rub lacking a citronella odor, but possessing a strong "cleaning agent" odor,

other factors unrelated to odors. Nevertheless, the apparent high rate of Africanized honey bee attacks on horses has led horse aficionados and veterinarians to speculate that fly repellents applied to horses might attract bees and increase the risk to horses and riders. The concern was especially true for those repellents containing citronella, a compound chemically related to the attractant Nasonov pheromone of honey bees (Schmidt et al. 1989). However, Collins et al. (1996) suggested that another common ingredient of insect repellents, DEET (N,N-diethyl-m-toluamide), was an effective repellent of attacking honey bees and might have potential for personal protection. The current investigation determined if DEET and common insect repellents or lotions used on horses potentially increased the likelihood or vigor of attacks by Africanized honey bees.

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Number of Strikes: Pyranha versus Control

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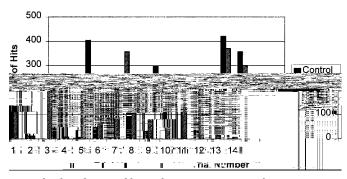


Fig. 1. Number of target strikes by Africanized honey bees in response to colony exposure to Pyranha-laden or control air.

and containing pyrethrins and piperonyl butoxide as active ingredients. The chemical odors were introduced to the colonies via air blown through a charged cartridge on the end of a 2-m × 1.25-cm diameter Teflon (United States Plastic, Lima, OH) tube connected to the exhaust port of a new, unused honey bee smoker. Multiple chemical cartridges were created from 5-cm sections of Teflon tubing, each with a filter paper disc, ≈0.5 cm in diameter, suspended near the middle with an insect specimen pin. Cartridges were prepared shortly before use by pipetting 5 μ l of test solution onto the filter paper and then the cartridge ends were sealed with rubber stoppers to minimize chemical loss. For testing, a cartridge was attached to the end of the smoker's Teflon tubing just before the test commenced, and odor-laden air was blown toward the hive entrance by compressing the bellows of the smoker. A data logging temper tester (Spangler and Sprenkle 1997) measured the number of attacking honey bees stimulated by the chemical essence. In brief, the temper tester consisted of a black plastic ellipsoid target ≈8 cm long and 5.5 cm in diameter containing a microphone attached by electrical cord to an electronic monitor that records honey bee strikes.

Each trial consisted of four random exposure tests, two with a test odor and two with an outside air negative control, for a single colony. Two trials of each test odor were conducted on each colony with the order of exposure reversed for the two trials of the colony to normalize the first-response variable. During the short, ≤30-s period when the experimenters were near the colonies, they were careful to hold their breath. During this time, the cartridge was positioned within 15 cm of the hive entrance, and the test commenced with 10 compressions of the smoker bellows, over an ≈10-s period. Upon completion of the compressions, the operators removed the smoker and calmly departed the area to as far as feasible, and limited unnecessary movement, to avoid extraneous stimulation of the honey bee colony. The ensuing attack response was monitored for 1 min and 50 s after the chemical challenge ended with the final compression of the bellows. A 5-min calming period was provided between tests within a trial. Sixteen trials were conducted for each test odor source: i.e., two trials at each of eight bee colonies. For two trials during the testing of each Pyranha and Repel X, the colonies were unresponsive to any stimulus and were eliminated,

Number of Strikes: DEET versus Control

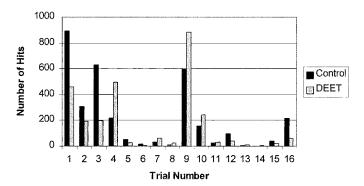


Fig. 2. Number of target strikes by Africanized honey bees in response to colony exposure to DEET-laden or control air.

Number of Strikes: Repel X versus Control

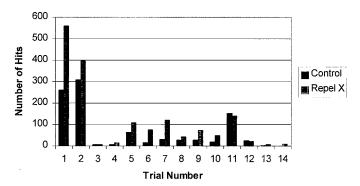


Fig. 3. Number of target strikes by Africanized honey bees in response to colony exposure to Repel X-laden or control air.

resulting in of only 14 replicates for both Pyranha and Repel X. A paired t-test compared the number of strikes stimulated by each chemical versus the concurrent controls (Zar 1996).

Results

Overall, Pyranha neither provoked nor repelled Africanized honey bee attacks (Fig. 1). In 6 of 14 trials (42.9%), honey bees exposed to Pyranha produced a higher number of strikes to the target than the unscented control. Bees struck after Pyranha exposure on average 126.7 ± 34.8 (SE) times per trial and 138.6 ± 42.4 for controls. The total number of strikes was 1,774 for Pyranha and 1,940 for the control (ratio of 0.91 Pyranha strikes per control strike) and did not differ significantly (P = 0.72).

Strikes recorded after exposure of colonies to DEET also was not significantly different from the odorless control (P=0.51) (Fig. 2). DEET stimulated less honey bee activity than the control in 8 of the 16 trials, with a mean number of respective strikes of 172.1 \pm 61.5 and 205.3 \pm 67.9 and total strikes of 2,753 and 3,285 (ratio of 0.84 DEET strikes per control strike), respectively.

Unlike Pyranha and DEET, Repel X exposure led to more temper tester strikes than controls in all but two trials (85.7%). In 8 of the 14 (57.1%) Repel X trials, the number of honey bees that made contact with the temper tester target was greater than twice that of the control. Average strikes per trial were 115.8 ± 43.6 for Repel X versus 67.6 ± 26.8 for controls, and he total number of strikes for Repel X, 1,621, was 1.71 times that for the control, 947. These differences were significant (P = 0.04) (Fig. 3).

Discussion

Africanized honey bees were neither stimulated nor subdued by DEET or Pyranha, whereas exposure to Repel X consistently provoked large numbers of attacking bees. These data do not necessarily contradict the claims made by Collins et al. (1996), that DEET effectively repels attacking honey bees; however, it does indicate that DEET had little or no effect on preventing the initiation of an attack. In other tests, Spangler et al. (1990) and Schmidt and Spangler (1991) showed that DEET might have a minor repellent affect against attacking bees, but the effect was substantially less than that produced by two aerosol insecticides and would likely be of little or no value in defending against a serious attack.

Pyranha contains citronella, an ingredient whose smell somewhat resembles that of the citral/geraniol/nerolic + geranic acid pheromone blend of honey bee Nasonov pheromone (Schmidt et al. 1989). Citronella and the Nasonov pheromone components are oxygenated monoterpenes and share chemical similarities. These similarities led to the hypothesis that horse rubs containing citronella might increase bee attacks on horses by attracting bees that mistake the rub for their own attractant Nasonov pheromone. Our data do not support this hypothesis and indicate no attractiveness by the citronella in Pyranha to attacking bees.

In contrast to Pyranha and DEET, the essence of Repel X consistently stimulated a larger attacking force of Africanized honey bees than unscented air controls. This was consistent with an increased probability of an Africanized honey bee attack with the use of this fly repellant on horses. The active ingredients, pyrethrins and piperonyl butoxide, are unlikely to stimulate a stronger attack response, because they are common to both Pyranha and Repel X. To the authors, Repel X produced a strong odor reminiscent of some household cleaning agents. Whether this odor or other inactive ingredients are responsible for the observed results is not known.

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